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PLAGUE VACCINE U.S.P.**

**Peter J. Bartelloni, et al**

**Army Medical Research Institute of Infectious  
Diseases  
Frederick, Maryland**

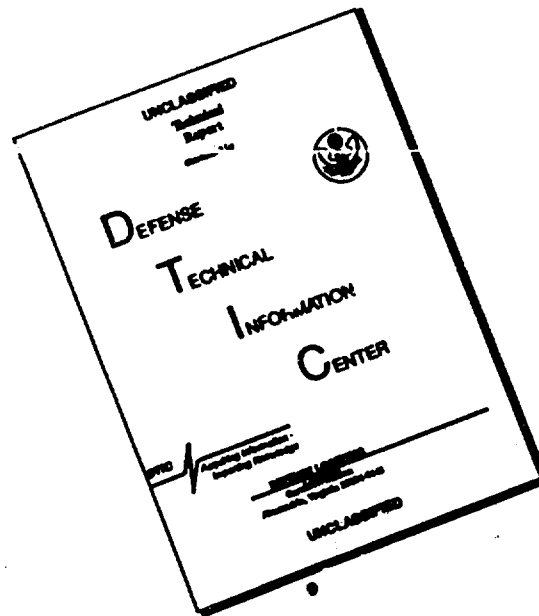
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# Clinical and Serological Responses to Plague Vaccine U.S.P.

LTC Peter J. Bartelloni, MC, USA†  
COL John D. Marshall, Jr., MSC, USA‡  
LTC Dan C. Cavanaugh, MSC, USA\*

In October 1941, the Subcommittee on Tropical Diseases, National Research Council Committee on Medical Research passed the following resolution: "Resolved that, even though the available knowledge does not seem to afford definite evidence of the benefits from the use of plague vaccines, it is advisable to vaccinate with killed plague bacilli of an approved strain all military or naval personnel under serious threat of exposure to bubonic plague."<sup>2</sup>

As a result, in 1942 a commercial biological laboratory began manufacture of a formal-killed plague vaccine under the guidance of Dr. K. F. Meyer.<sup>22</sup> During the next 29 years, the production of killed plague vaccine continued with minor modifications in the medium and strain of *Pasteurella pestis* (*Yersinia pestis*).

Studies pertaining to the manufacture, testing, and standardization of the vaccine have been reviewed elsewhere.<sup>3,4</sup> Meyer<sup>5</sup> reported on the antigenicity and reactogenicity in 25 humans administered the basic inoculation and one booster dose of Plague Vaccine U.S.P.

In the present study, volunteers were vaccinated according to the recommended schedule<sup>6</sup> and their sequential serological patterns of response were determined throughout a time-frame representative of a typical period of military service in a known plague area. Clinical responses were studied following the basic inoculation and each of two booster injections.

## Materials and Methods

### Vaccine

Plague Vaccine U.S.P. (E. Medium), Lots K1441 and K5240, employed in this study were produced by Cutter Laboratories, Berkeley, Calif., and were obtained through Army Medical Supply. The vaccine contained two billion formaldehyde-killed *Pasteurella pestis* per ml in Sodium Chloride for Injection, U.S.P., 0.04 per cent formalin, and trace amounts of: beef heart extract, yeast extract, agar and peptones and peptides from soya and casein. Phenol (0.5 per cent) was present as a preservative.

### Subjects

Twenty-nine healthy male volunteers between the ages of 18 and 25 not previously immunized with plague vaccine and having no history of infection with this bacterium were

studied on an outpatient basis. On day 0 each subject was administered 1.0 ml of plague vaccine intramuscularly (IM) in the deltoid region of the arm. On days 90 and 270, 0.2 ml booster doses of the vaccine were administered IM. Twenty-four and 48 hours after each dose of vaccine, the volunteers were observed for local and systemic reactions; oral temperatures were recorded each time.

### Serological Procedures

Blood was obtained from all persons for indirect hemagglutination (HA) and complement fixation (CF) tests before immunization and at varying intervals through day 390. All serum specimens were stored at 4°C and tested for CF and HA antibodies within 72 hours of collection and again at completion of the study with the microtiter technique of Cavanaugh *et al.*<sup>1</sup> Fraction 1 antigen of *P. pestis* was used in both tests. The HA test employed sheep red blood cells (SRBC) stabilized with pyruvate aldehyde and sensitized by coupling the Fraction 1 to SRBC with chromic chloride.

Mouse protective antibody indices (MPI) were determined by Dr. Meyer on sera from a selected group of individuals.<sup>6</sup> The MPI is determined by intravenous inoculation of 0.5 ml of the test serum into each of 10 mice, immediately followed by administration of 3,000 median lethal doses (LD<sub>50</sub>) of *P. pestis* 195/P subcutaneously. The MPI is calculated by dividing percent mortality by average day of death. An MPI of less than 10 is an indication of immunity.<sup>5</sup>

## Results

### Clinical Observation

Reactions (Table I) varied from none to mild, the latter characterized by soreness, erythema and induration at the site of inoculation. Following the initial dose of vaccine, 25 of the 29 vaccinees experienced soreness, two of whom developed erythema two to five mm in diameter. After the second dose of vaccine, 19 individuals complained of soreness, of whom five developed erythema two to 20 mm in diameter. Three of the 19 developed induration two to eight mm in diameter. Among the volunteers not experiencing

TABLE I  
REACTIONS OF 29 VOLUNTEERS TO THE ADMINISTRATION  
OF PLAGUE VACCINE U.S.P. (E MEDIUM)

Day of Inoculation	No Reaction	Mild Reactions
0	4	25
90	8	21
270	13	16

From the US Army Medical Research Institute of Infectious Diseases, Frederick, Md. 21701.

†Present address, Box 301, Tripler Army Medical Center, APO San Francisco 96438.

‡US Army Research and Development Group (Far East), APO San Francisco 96343.

\*Walter Reed Army Institute of Research, Washington, D.C. 20012.

\*\*Director Emeritus, G. W. Hooper Foundation for Medical Research, Univ. of California, San Francisco Medical Center, San Francisco, Calif.

soreness, one developed erythema and another, erythema and induration.

Following the last dose of vaccine, 15 subjects experienced soreness, one of whom developed erythema (10 mm in diameter). An additional vaccinee developed only erythema. These reactions persisted for less than 48 hours. No subject developed an oral temperature of 99.4°F or higher during the period of observation. No systemic reactions were observed in any of the vaccinees.

### Serological Evaluation

The serological results are summarized in Table II. Two of 29 subjects had an HA antibody titer of 1:8 in their pre-immunization test sera; however, they responded adequately to the vaccine. Examination of the medical records and detailed questioning of these two individuals failed to elicit a history of prior plague immunization or travel in enzootic plague areas. In response to the initial 1.0 ml dose of vaccine, 24 of the 29 subjects (83 per cent) produced detectable HA antibodies by day 15 and an additional individual converted by day 30. By day 90, the HA titers of these 25 subjects had decreased. CF antibody was not demonstrated in any of the sera collected through day 90.

An immediate booster response as determined by the HA test was evident in 26 individuals (90 per cent), 15 days after the second inoculation of 0.2 ml of plague vaccine (day 105). There was a steady decline in both the number of subjects with detectable HA antibody and in individual HA titers throughout the following 180 days. Low CF antibody titers that were demonstrated in the sera of 19

subjects (66 per cent), 15 days after booster immunization, disappeared in all but one person by day 270.

The second booster dose of 0.2 ml of vaccine stimulated production of HA antibodies in 27 individuals (93 per cent) and CF antibodies in 17 (59 per cent). Individual titers were higher than those resulting from either of the previous injections. Whereas there was a gradual decrease in the titer of most individuals during the subsequent four-month period, the rate of decline was relatively slower than that observed after the first two doses of vaccine. This trend toward stabilization of HA antibody titer was not observed with CF antibody. Two individuals failed to produce detectable HA antibodies, despite the administration of three doses of vaccine.

Presented in Table III is a summary of HA, CF and MPI values for 10 selected individuals, half of whom showed poor serological response as measured by the HA test and half, significant HA titers after the primary inoculation. The results show a good correlation between high HA titers and low MPI values. CF antibody, when present, always was accompanied by high HA titers and low MPI values, although the reverse was not necessarily true.

### Discussion

Reactions to the administration of plague vaccine in a three-dose schedule were mild and consisted only of soreness at the site of inoculation, erythema, and/or induration. Such reactions persisted for 24 to 48 hours. There were no systemic reactions. It has been reported that, with repeated doses of vaccine, systemic reactions such as fever, headache, and malaise may occur more often and be more severe.<sup>7</sup> Allergic reactions, manifested primarily by varying degrees of urticaria, as observed by Reisman,<sup>8</sup> were not seen in our group of vaccinees.

The serological results were essentially in agreement with the observations reported by Meyer.<sup>5</sup> The major difference was the CF antibody response in 55 per cent of our subjects 30 days after the first booster dose of vaccine, as compared with the absence of detectable CF antibody in Meyer's study.

The failure of two of 29 individuals (seven per cent) to produce detectable antibodies even after a second booster dose is cause for some concern. The relationship in man between immunity to plague and humoral antibodies as measured by the HA, CF or MPI tests is unknown. However, based on challenge experiments in a variety of animal species, a high degree of correlation has been observed between protection against fatal infection and the presence of detectable antibody. In our study, tests on the sera of selected individuals showed that those having an HA titer of 1:128 or greater had MPI values of 10 or less. Conversely, those individuals with low or undetectable HA antibody titers had MPI values of 12 or above. Based on this relationship, an 83 per cent conversion rate was obtained with two immunizations. The results of our study indicate that some individuals fail to develop a serological response to the current immunization program. This fact should serve to alert medical personnel in endemic areas of the possibility of encountering plague not only in the native population but also among troops.

TABLE II  
HEMAGGLUTINATION AND COMPLEMENT-FIXING ANTIBODY RESPONSES  
OF 29 INDIVIDUALS ADMINISTERED PLAGUE VACCINE U.S.P.  
(E MEDIUM)

Vaccine Dose ml	Day	HA Positive*			CF Positive**		
		No.	%	GMT†	No.	%	GMT
1.0	0	2	7	0.2	0		
	15	24	83	25	0		
	30	25	86	22	0		
	60	25	86	27	0		
0.2	90	24	83	19	0		
	105	26	90	140	19	66	6.0
	120	26	90	106	16	55	4.0
	150	26	90	96	7	25	1.5
	180	25	86	67	3	10	0.7
	210	24	83	48	2	6	0.5
	240	24	83	44	1	3	0.2
	270	23	79	24	1	3	0.2
0.2	285	27	93	576	17	59	6.5
	300	27	93	383	17	59	5.2
	330	27	93	320	15	52	2.9
	360	26	90	314	3	10	0.9
	390	21/25	84	295	1	4	0.2

\*An HA titer  $\geq 1:32$  was considered positive.

\*\*A CF titer  $\geq 1:4$  was considered positive.

†Geometric mean titer.

TABLE III  
ANTIBODY RESPONSES AND MOUSE PROTECTIVE INDICES OF  
10 SELECTED VOLUNTEERS GIVEN THREE DOSES OF  
PLAGUE VACCINE U.S.P. (E MEDIUM)

Day	Geometric Mean Reciprocal Titer				Mouse Protective Index	
	HA	(No. > 1:32)	CF	(No. > 1:4)	Arithmetic Mean	(No. < 10)
Group I:						
Low Reactors (5)						
0 Prebasic	0	(0)	0	(0)	18	(0)
30 Postbasic	<1	(0)	0	(0)	16	(0)
90 Prebooster	<1	(0)	0	(0)	16	(0)
120 Postbooster	<1	(2)	0	(0)	16	(0)
270 Prebooster	<1	(0)	0	(0)	18	(0)
300 Postbooster	1	(3)	<1	(1)	16	(0)
Group II:						
High Reactors (5)						
0 Prebasic	<1	(0)	0	(0)	20	(0)
30 Postbasic	64	(5)	0	(0)	12	(1)
90 Prebooster	74	(5)	0	(0)	11	(2)
120 Postbooster	512	(5)	18	(5)	3	(5)
270 Prebooster	147	(5)	<1	(1)	5	(5)
300 Postbooster	2690	(5)	28	(5)	4	(5)

### Summary

Twenty-nine volunteers were administered a basic inoculation and two booster doses of plague vaccine. Reactogenicity was determined following each dose of vaccine, and sera for serological tests were collected at selected intervals over a period of 390 days. The vaccine was found to be of low reactogenicity. Thirty days after the initial dose of vaccine, 86 per cent of the subjects produced detectable HA antibodies. Subsequent booster doses of vaccine stimulated increased production of HA antibody, which persisted

longer with each successive dose. Individuals developing an HA antibody titer of 1:128 or greater had MPI values of 10 or less. CF antibody in low titer developed following the first and second booster doses of vaccine in 19 per cent and 17 per cent, respectively, of subjects but CF titers diminished rapidly. Two individuals failed to produce HA antibody, even after the three doses of vaccine.

### Acknowledgments

This study was conducted as a portion of a comprehensive investigation concerning the prevention and therapy of infectious diseases and was supervised by the Commission on Epidemiological Survey of the Armed Forces Epidemiological Board. All details and purposes of this study were carefully explained in advance to each individual who volunteered (US Army Regulation 70-25, Use of Volunteers as Subjects of Research, Department of the Army, Washington, D.C., 1962 and the Declaration of Helsinki). The cooperation of the National Service Organization, General Conference of the Seventh-Day Adventists is gratefully acknowledged.

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